

Amendments to the Specification:

Please replace paragraph [0088] beginning at page 28, line 29, with the following:

--[0088] A fluorescence polarization competition assay was used to detect binding of the compounds to MAGI-3 PDZ2. A fluorescein labeled carboxy terminal sequence of PTEN, OregonGreenTM-PFDEDQHTQITKV-COOH (SEQ ID NO:1), was used as a probe. For a positive control, we chose PFDEDQHTQITWV-COOH (SEQ ID NO:2), the highest affinity peptide sequence for MAGI-3 PDZ2 known. To synthesize the labeled peptide we used standard Fmoc conditions on Wang resin to build a 13 residue peptide. A typical coupling cycle includes deprotecting the terminal amino acid with 20% piperidine in dry DMF, washing the resin 2-3 times with DMF then methylene chloride and both again, and determining the existence of free amine by ninhydrin kaiser test. A slurry containing coupling reagent 2.4 equivalents of HBTU, the next N terminal amino acid to be added to the growing peptide 2.5 equivalents of Fmoc protected amino acid, 5 equivalents of DIEA in dry DMF. The amino acid was coupled over 2-3 hours and the kaiser test was used to determine completeness. Coupling steps were repeated if a positive kaiser test resulted. This method was used for each residue of the peptide. The finished peptide was cleaved from the resin with 95% TFA with a cocktail of scavengers including thioanisole and phenol. The peptide was precipitated with ether and lyophilized. Peptides were purified using HPLC and identified with MALDI mass spectrometry.--

Please replace paragraph [0090] beginning at page 29, line 21, with the following:

--[0090] Figure 2 shows the fluorescence polarization competition of inhibitor 3 (above) (Table I, compound 1) for the binding site of OG-PFDEDQHTQITTV-COOH (SEQ ID NO:3) (10 nM) on GST-MAGI-3 PDZ2 (300 nM).--

Please replace paragraph [0093] beginning at page 30, line 2, with the following:

--[0093] ~~Anti-phospho-Ser473 for immunoblotting were produced by injecting rabbits with the peptides CRPHFPQFS(P)YSASGT, and antibodies recognizing unphosphorylated peptide~~ Anti-phospho-Ser473 for immunoblotting were produced by injecting rabbits with the peptides CRPHFPQFS(P)YSASGT (SEQ ID NO:4), and antibodies recognizing unphosphorylated peptide were removed by binding to nonphosphopeptide columns. The unbound material was then affinity purified over a phosphopeptide column. The antibodies used for PKB immunoprecipitation (IP) kinase assays were generated by injecting rabbits with recombinant full-length PKB. Polyclonal anti-SHIP-2 antibody was generated by immunizing rabbits with glutathione S-transferase fused to the C-terminal region of SHIP-2. Anti-PKB kinase (i.e., PDK-1) was purchased from Transduction Laboratories. Horseradish peroxidase-conjugated secondary antibodies were obtained from Amersham-Pharmacia.--

Please replace paragraph [0094] beginning at page 30, line 14, with the following:

--[0094] Subconfluent monolayers of HCT116 cells were lysed by scraping the cells into lysis buffer (20 mM Tris-HCl, pH 7.5, containing 1% NP-40, 1 mM EGTA, 1 mM EDTA, 1 mM sodium orthovanadate, and protease inhibitor cocktail [Boehringer Mannheim]) at 4°C. After centrifugation (10,000 x g for 10 min at 4°C) to remove insoluble components, endogenous PKB was immunoprecipitated (IPed) using the anti-PKB antibody and protein A-Sepharose at 4°C for 1 h. After washing the IP, kinase activity was assayed using the synthetic peptide GRPRTSSFAEG (SEQ ID NO:5) (Crosstide) as a substrate in a reaction mixture containing 20 mM Tris-HCl (pH 7.5), 75 mM NaCl, 10 mM MgCl₂, 1 mM dithiothreitol (DTT), 20 μM ATP, 50 μM Crosstide, and 5 μCi of [³²P]ATP in a volume of 20 μl per assay. The reaction was allowed to proceed for 15 min at 30°C and then was stopped by spotting 18 μl onto Whatman P81 filter papers and immersing them in 1% (vol/vol) orthophosphoric acid. The papers were washed four times, rinsed once in acetone, and air dried, and the radioactivity was determined by

Appl. No. 10/826,175

PATENT

Amdt. dated July 13, 2004

Reply to Notice to File Missing Parts of June 28, 2004

scintillation counting. Alternatively, the phosphorylation reactions were stopped by the addition of Tricine sample buffer, the phosphopeptide was separated on a 16% Tricine gel, and the amount of ^{32}P radioactivity was assessed using a STORM PhosphorImager (Molecular Dynamics).--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 and 2, at the end of the application.